THE RESPIRATORY FUNCTION OF THE BLOOD OF MARINE FISHES

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Introduction

The material embodied in this paper is a report of a study of marine fish blood from the standpoint of respiratory function. Since we are now fairly cognizant of the rôle of blood in mammals, it seemed to the author that the scope of investigation should be widened by a study of species other than mammals. In choosing marine fishes as experimental material the writer had not only this point in mind, but, in addition, the thought that fishes might present some new and interesting aspect in blood physiology because of the fact that their method of blood aëration is quite different from that of mammals. The blood of mammals is apparently adjusted to the environment offered by the alveoli of the lungs where high carbon dioxide tensions prevail and oxygen tensions lower than in air exist. On the other hand, the gill of a fish is bathed in a medium where higher oxygen tensions and much lower carbon dioxide tensions prevail than is the case in the lung of a mammal. In addition to these interesting differences, fish bloods possess nucleated, instead of non-nucleated, red corpuscles, variable quantities of hemoglobin (Hall and Gray, 1929), and function in varying, rather than constant, temperatures.

There is little work on the respiratory function of fish blood to be found in the literature. Trendelenburg (1912), Gaarder (1918), Krogh and Leitch (1919), Nicloux (1923), and Wastl (1928) have investigated the blood of fishes. Krogh and Leitch found a distinct difference between oxygen dissociation curves for the bloods of the fresh-water fishes, carp, pike, and eel, and the marine cod and plaice. According to them, the hemoglobin of both types of fishes is very sensitive to carbon dioxide, and the characteristics of their blood, as far as the transportation of oxygen is concerned, are adjusted to the environment in which the fishes are living. Wastl has published oxygen dissociation curves, carbon dioxide absorption curves, and figures for arterial gas content and hydrogen ion concentration of carp blood. Distinct differences were found between the blood of the carp and that of mammals. Jolyet and Regnard (1877), and Kawamoto (1929) have studied the blood of the eel. Kawamoto determined the relationship

between the oxygen dissociation of the hemoglobin and temperature. Collip (1920), Powers (1922), Jobes and Jewell (1927), and Kokubo (1927, 1930) have investigated the alkaline reserve of several fishes. Hall and collaborators (1926, 1928, 1929) have published data for the hemoglobin concentration of the blood of a number of marine species.

The investigation to be reported in this paper has been restricted for the most part to determinations of the oxygen capacities, oxygen dissociation curves, carbon dioxide absorption curves, the effect of carbon dioxide on the oxygen capacity, and the buffering capacities of the bloods. The general results have been compared with similar results obtained by other investigators on other vertebrates. The experimental work was carried on at Woods Hole, Massachusetts, in the laboratory of the United States Bureau of Fisheries.

Methods

Experimental Animals.—The fishes that were employed in the study are species common to the region of Woods Hole, Massachusetts. Three species furnished most of the results, namely, the toadfish, *Opsanus tau* (Linnaeus), the sea robin, *Prionotus carolinus* (Linnaeus), and the common mackerel, *Scomber scombrus* (Linnaeus). Some work was also done on the goosefish, *Lophius piscatorius* (Linnaeus), the scup, *Stenotomus chrysops* (Linnaeus), and the puffer, *Spheroides maculatus* (Bloch and Schneider). The fishes were maintained at the laboratory under conditions as nearly normal as possible by keeping them in "live-cars" or in hatching-boxes where plenty of running seawater was supplied at all times. The importance of keeping them in good condition has been aptly pointed out by Hall, Gray, and Lepkovsky (1926).

The choice of the three fishes, the toadfish, sea robin, and mackerel requires some explanation. Hall and Gray (1929), and Gray and Hall (1930) have made a study of the blood sugar, hemoglobin, and iron of these fishes and found a fairly precise correlation between these factors and the activity of the fishes. The mackerel, for example, is an active fish and is characterized by a high concentration of sugar, iron, and hemoglobin in its blood, while the toadfish is a sluggish fish and is characterized by a low concentration of blood sugar, hemoglobin, and iron. The sea robin is more or less intermediate in this respect. On the basis of this information it seemed worthwhile to broaden the study enough to include several "type" fishes, instead of restricting observations to only one type. Another factor of a more practical turn was influential in the choice of these fishes. The blood of fishes does

not lend itself easily to gas analysis. This has been recognized by others, and is probably one reason why more work has not been done. On account of the small size of many fishes, blood is not easily obtained for study. Some fishes have very fragile red corpuscles which makes it almost impossible to subject their blood to the drastic treatment necessary in determining dissociation curves. Also fish blood reacts peculiarly toward the reagent, potassium ferricyanide, used to liberate oxygen. As soon as the reagent comes in contact with the blood a coagulum is formed. Under these conditions it is quite impossible to liberate all the oxygen from the blood without subjecting it to vigorous, prolonged shaking. The blood from the fishes employed reacts no differently from other fish bloods toward ferricyanide, but is quite suitable in other respects. This is especially true of toadfish and sea robin bloods. Mackerel blood is quite viscous and makes pipetting rather annoying. It is also the hardest of the three to handle in the Van Slyke extraction chamber, for its coagulum adheres to the walls and is not easily cleaned out.

Obtaining of Blood Samples.—In obtaining blood for analysis an attempt was made to standardize conditions as much as possible. When it was not desired to know the gas content actually existing in the blood at the time of drawing, the procedure was to remove a fish quickly from the water and bleed it from the gills by means of a hypodermic needle attached to a 5 or 10 cc. syringe. Lithium oxalate was used as an anticoagulant. The time of bleeding was made as short as possible in order to avoid getting blood that might have excess acid in it on account of asphyxial conditions. Hall (1928) has shown that asphyxia in fishes lowers the oxygen capacity of their blood considerably.

Since most of the fishes used were small, it was found necessary to combine the blood of several specimens of a species. This practice led to no ill effects. In fact, the analytical results on different blood specimens checked more closely than otherwise on account of the averaging effect of such a procedure.

The blood was used as soon as it was drawn. In preliminary work addition of both sodium fluoride and potassium cyanide to the blood to prevent respiration of the cells and loss in carbon dioxide-combining power was tried. The results were unsatisfactory. The slight loss in carbon dioxide-combining power over a period of time did not appear to be checked. Rather than add more extraneous chemical factors, it was finally decided to modify the procedure in such a way as to avoid any appreciable error due to the activity of the cells. This necessitated using a given sample of blood a shorter length of time and checking a curve that had once been established by means of freshly drawn blood.

It also made it necessary that a blood sample be analyzed for its gas content as soon as it had come into equilibrium with a given gas tension, and that the gas phase be separated from the blood remaining in the tonometer during the time consumed in the analysis. It should be mentioned at this time that Dr. F. G. Hall (unpublished) has determined the oxygen consumption of these bloods and shown, under the conditions of the author's technique, that the error arising from oxygen consumption of the cells would be negligible over the short period of time that elapses in getting a blood sample into the Van Slyke apparatus from the tonometer.

When it was desired to know the actual content of gases existing in the blood at the time of drawing, the method was modified to suit the purpose. In attempts to determine arterial or venous gas contents, fishes were placed in suitable traps and a stream of fresh sea-water directed over their gills. The blood was then drawn under oil and the gases immediately analyzed. It is most difficult to get a satisfactory technique for determining arterial and venous gases in fishes. The results obtained are only approximate at best.

Determination of Erythrocyte Count and Volume.—The number of red corpuscles per cubic millimeter of blood was determined by employing the usual procedure. The volume of red corpuscles was determined by an haematocrit especially designed by Dr. F. G. Hall for use with fish blood.

Equilibration of Blood with Gases and Determination of Gases.—The gases used in these experiments were carbon dioxide, oxygen, and nitrogen. The required mixtures were made in a mixing chamber attached to an ordinary gas burette (if gas mixtures different from air were desired). The method of handling the blood and gases was essentially the same as that prescribed by Austin *et al.* (1922), except for the admittance of gases to tonometers. Instead of using the method they prescribe, the tonometers were filled with clean, neutral mercury, and the gas mixtures drawn into them from the mixing chamber by withdrawing the mercury. The equilibration of blood samples was carried out according to their "first saturation method," using the double tonometer. Equilibration for all samples was allowed to take place at 20° C. and at atmospheric pressure. Atmospheric pressure was maintained by occasionally opening the stop-cock on the tonometer. Since the gases in the tonometer were always analyzed after equilibration, the entrance of a small amount of gas from the atmosphere did no harm. The tonometers were mechanically rotated in a thermostatically controlled water bath for a period of about 30 minutes. It was found in preliminary experiments that this was sufficient time to allow the blood

and gas phase to come into equilibrium with each other. Usually one tonometer was rotated at a time. However, in some of the work involving carbon dioxide absorption, two tonometers were used simultaneously, one containing reduced and the other oxygenated blood.

At the end of equilibration a sample of blood was removed from the tonometer and the gases in it immediately analyzed according to the technique of Van Slyke and Neill (1924). Both oxygen and carbon dioxide were simultaneously liberated from the blood by using acid ferricyanide. One cubic milliliter of blood was used for each analysis, and was admitted to the extraction chamber by means of a Van Slyke differential pipette. The blood was agitated in the extraction chamber a little longer than is usual for mammalian blood. This was found necessary in order to insure the complete liberation of gases. Both the carbon dioxide and oxygen were absorbed after liberation, sodium hydroxide being used for carbon dioxide, and sodium hydrosulfide for oxygen.

TABLE I

Oxygen capacity determinations. Blood equilibrated with air at 20° C.

Species	Oxygen capacity	Red Blood Corpuscles	Hæmatocrit	Iron
	vol. per cent	*cu. mm.*	*vol. per cent*	*mg. 100 cc.*
Goosefish	5.07	867,083	15.45	13.40
Toadfish	6.21	585,000	19.50	14.00
Puffer	6.75	2,284,000	17.50	21.50
Scup	7.30	2,685,000	32.60	24.60
Sea robin	7.66	2,536,000	24.00	23.10
Mackerel	15.77	3,000,000	37.10	37.10

The amount of carbon dioxide and oxygen in blood was expressed as volumes per cent of dry gas at 760 mm., and 0° C., the tables prepared by Van Slyke and Neill (1924) being used for oxygen, and those prepared by Van Slyke and Sendroy (1927) for carbon dioxide. In determining the oxygen combined with hemoglobin, the amount of oxygen physically dissolved was calculated on the basis of Bohr's (1905) solubility coefficients. A special equation similar to that of Peters, Bulger, and Eisenman (1923) was employed in the calculation to allow for the variable corpuscular volume in the various bloods.

The concentration of the gaseous phase in the tonometers was determined after equilibration of blood samples by analysis in the Haldane apparatus as modified by Henderson (1918). The results were expressed in terms of tension by employing the usual calculations.

Method of Studying Lactic Acid Effect.—Lactic acid was carefully added to small samples of blood in amounts necessary to give the de-

sired concentration. The blood was then equilibrated in air and handled the same as in the other experiments.

Calculation of pH of Blood.—In calculating the pH of fish blood the familiar Henderson-Hasselbalch equation was used (Henderson, 1908; Hasselbalch, 1917). A pK′ factor of 6.24 was employed for the blood at 20° C. This was derived by using the average pK′ factor of 6.13 that has been worked out for mammalian serum at 38° C. (using Bohr's, 1905, solubility coefficient for CO_2) by a series of workers (Warburg, 1922; Cullen, Keeler, and Robinson, 1925; Van Slyke, Hastings, Murray, and Sendroy, 1925; and Hastings, Sendroy and Van Slyke, 1928), and adding a temperature correction of 0.005 for each degree below

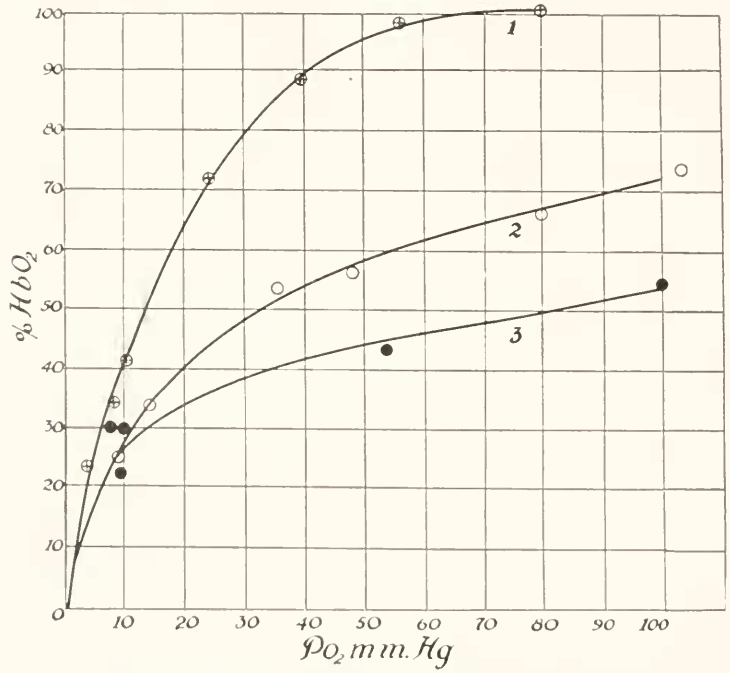

FIG. 1. Oxygen dissociation curves for toadfish blood at 20° C. Curve 1 at 1 mm. carbon dioxide; curve 2 at 10 mm. carbon dioxide; and curve 3 at 25 mm. carbon dioxide tension.

38° C. (Hasselbalch, 1917; and Warburg, 1922). In addition a correction of 0.02 was added because whole blood was used instead of serum. The pK′ factor for whole blood is slightly higher than that for serum (Warburg, 1922; Peters, Bulger, and Eisenman, 1923; and Van Slyke *et al.*, 1925).

In using the pK′ factor in the following calculations of pH, it is recognized that there are many variables which enter into its composi-

tion for any one blood, especially when it is applied to whole blood. Warburg (1922), Hastings and Sendroy (1925), Stadie and Hawes (1928), and Stadie (1928) have shown that the pK′ factor is affected by the ionic strength of the solution in which it is measured. Furthermore, the researches of Warburg (1922), Van Slyke, Wu and McLean (1923), and Peters, Bulger, and Eisenman (1923) have demonstrated the effect of degree of oxygenation of blood, its pH, and its relative volume of corpuscles and plasma upon the pK′ factor.

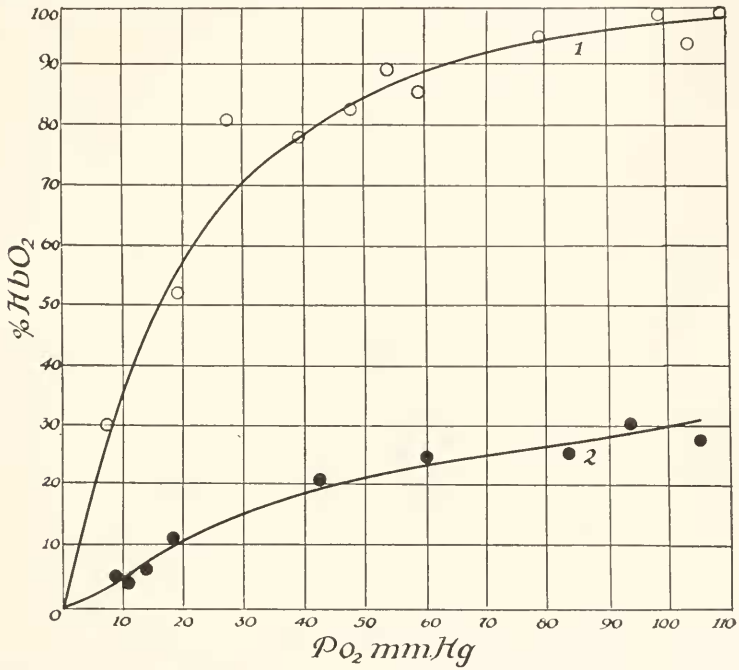

FIG. 2. Oxygen dissociation curves for sea robin blood at 20° C. Curve 1 at 1 mm. carbon dioxide; and curve 2 at 25 mm. carbon dioxide tension.

However, there is little information at the present time that will permit the calculation of the pH of fish blood with the degree of refinement that now seems possible for mammalian blood. Therefore the author does not claim absolute accuracy for the calculated pH of fish blood, but only relative, and admits that with the advent of more information his figures will probably require correction.

RESULTS

A. The Transportation of Oxygen

Oxygen Capacity of Blood.—The results of this study are summarized in Table I. The figures for oxygen capacity are those obtained when the blood was equilibrated in air, and dissolved oxygen subtracted. Thus they represent the actual amount of oxygen combined

with hemoglobin under the conditions of the experiment. An attempt has been made to correlate oxygen capacities of the various bloods with their corpuscle count, corpuscle volume, and iron content. The author is indebted to Dr. F. G. Hall and Mr. S. R. Tipton for some of the data contained in the last three columns of Table I. It should be mentioned

TABLE II

Gases in Blood, as Drawn under Oil

Species	Kind of Blood	CO_2	O_2	P_{CO_2}	P_{O_2}	HbO_2	Conditions of Drawing
		vol. per cent	*vol. per cent*	*mm. Hg.*	*mm. Hg.*	*per cent*	
Scup	Arterial	8.15	8.33	—	—	—	Water over gills Blood from gills
		9.16	5.00	—	—	59	Water over gills Blood from caudal artery
		8.90	5.24	—	—	69	Water over gills Blood from gills
		11.40	3.13	—	—	—	Water over gills Blood from gills
Sea robin		6.15	2.55	2	10	33.2	Water over gills Blood from gills
Toadfish	Venous	13.30	0.54	10	2	7.6	Water over gills Blood from heart
Goosefish		10.25	Trace	—	—	—	Water over gills Blood from bulbus
Puffer		14.90	0.34	—	—	5.3	Water over gills Blood from sinus venosus
Sea robin	Asphyxial	9.08	2.41	4	20	31.5	Fish in air Blood from gills
		13.40	1.59	10	20	22.2	Fish in air Blood from gills

that the figures for corpuscle count, corpuscle volume, and iron content were not always obtained from the same samples of blood on which oxygen capacity determinations were made. The data represent the average of a considerable number of determinations. There appears to be a general correlation between the oxygen capacity of fish blood and the previously mentioned factors. The best agreement exists between iron and oxygen. Since the corpuscle count and volume are variable among themselves, on account of differences in size of corpuscles, these factors do not show as good a correlation as iron.

The most interesting feature of this phase of the work is that it points out great differences in the oxygen capacities of the various bloods. The sluggish goosefish and toadfish possess bloods of low oxygen capacity, whereas the active mackerel has a blood of high oxygen capacity.

TABLE III

Oxygen dissociation of blood. Equilibrated at 20° C.

Species	P_{CO_2}	P_{O_2}	O_2-Capacity	O_2-Content	O_2-Dissolved	O_2 Combined	HbO_2	pH
	mm. Hg	*mm. Hg*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	
Toadfish	0.762	3.75	6.84	1.56	0.015	1.54	22.5	7.86
	0.454	7.80	5.13	1.75	0.030	1.72	33.5	7.99
	1.150	10.30	6.31	2.66	0.040	2.62	41.6	7.68
	0.615	24.60	5.13	3.78	0.096	3.68	71.8	7.60
	0.765	39.20	5.13	4.70	0.152	4.55	88.3	7.78
	0.690	56.20	5.13	5.28	0.219	5.06	98.6	7.70
	0.690	80.00	5.13	5.51	0.312	5.20	101.4	7.66
	8.62	8.85	6.31	1.65	0.035	1.62	25.6	7.33
	10.85	13.35	6.68	2.37	0.052	2.32	34.6	7.21
	11.15	35.70	6.68	3.64	0.138	3.50	52.3	7.18
	11.25	48.20	6.68	4.00	0.188	3.81	57.0	7.16
	10.42	80.00	6.68	4.75	0.312	4.44	66.4	7.17
	10.28	103.00	6.68	5.25	0.400	4.85	72.6	7.16
	25.40	7.47	5.56	1.63	0.029	1.60	28.8	6.98
	25.10	9.63	6.31	1.43	0.037	1.37	22.0	7.00
	27.40	10.00	6.84	1.93	0.039	1.89	27.5	6.94
	25.80	53.00	5.56	2.62	0.207	2.41	43.5	6.97
	25.05	100.00	5.56	3.38	0.390	2.99	53.8	6.98
Sea robin	0.304	6.69	7.02	2.15	0.026	2.12	30.2	8.22
	0.485	19.40	7.80	4.09	0.075	4.02	52.1	8.16
	0.227	27.00	8.20	6.72	0.100	6.62	80.7	8.03
	0.727	39.70	6.82	5.47	0.155	5.32	78.0	7.86
	1.050	47.20	7.91	6.70	0.184	6.52	82.5	7.67
	0.455	54.50	8.20	7.55	0.212	7.34	89.5	7.67
	1.510	59.70	7.25	6.40	0.233	6.17	85.1	7.68
	1.160	79.00	7.25	7.22	0.308	6.91	95.3	7.43
	1.132	99.00	7.91	8.19	0.386	7.80	98.6	7.13
	0.761	104.50	7.04	6.94	0.408	6.53	93.0	7.83
	0.225	109.00	7.20	7.50	0.425	7.08	98.3	8.09
	24.70	9.35	7.66	0.42	0.036	0.38	6.3	7.05
	28.10	10.75	7.10	0.32	0.042	0.28	4.0	6.98
	25.00	13.50	6.85	0.50	0.053	0.45	6.5	7.03
	26.10	17.00	7.15	0.85	0.066	0.78	11.0	7.00
	24.40	17.80	6.97	0.89	0.069	0.82	11.6	7.04
	21.80	18.20	7.15	0.83	0.071	0.76	10.8	7.13
	25.10	42.00	7.15	1.61	0.164	1.45	20.2	7.06
	25.60	60.50	7.15	1.94	0.236	1.70	23.8	7.04
	26.00	84.50	6.97	2.12	0.330	1.79	25.6	7.05
	25.90	92.50	7.15	2.50	0.360	2.14	30.0	7.00
	23.60	106.50	6.97	2.36	0.415	1.95	28.0	7.12

TABLE III—*Continued*

Species	P_{CO_2}	P_{O_2}	O_2-Capacity	O_2-Content	O_2-Dissolved	O_2 Combined	HbO_2	pH
	mm. Hg	*mm. Hg*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	
Mackerel	1.130	4.74	16.41	1.76	0.018	1.74	11.0	8.19
	1.250	8.30	15.76	3.46	0.032	3.43	21.6	8.00
	0.640	17.70	14.72	8.11	0.067	8.04	53.9	8.17
	0.977	31.95	16.29	12.45	0.122	12.33	75.8	7.96
	0.754	45.00	16.64	13.60	0.172	13.43	80.7	8.03
	0.382	64.60	17.81	16.35	0.247	16.10	90.43	8.00
	0.825	75.60	16.64	15.30	0.290	15.01	90.2	7.86
	0.768	98.70	15.59	14.75	0.378	14.37	92.2	7.48
	0.758	115.00	16.64	16.10	0.440	15.66	94.1	7.60
	10.17	9.87	14.54	1.01	0.038	0.97	6.7	7.49
	10.10	14.50	14.54	2.15	0.055	2.10	14.5	7.58
	11.30	29.40	14.54	4.13	0.113	4.02	27.7	7.51
	10.45	46.80	14.54	7.15	0.179	6.97	48.0	7.51
	11.00	65.00	14.54	7.70	0.249	7.45	52.0	7.49
	10.30	77.50	14.54	9.82	0.297	9.52	65.5	7.43
	10.35	83.30	14.54	10.30	0.319	9.98	68.6	7.46
	10.85	101.00	14.54	11.00	0.387	10.61	73.0	7.42
	24.50	12.00	17.81	0.92	0.046	0.87	5.0	7.33
	18.85	40.50	16.62	6.35	0.155	6.20	37.3	7.36
	24.80	53.40	16.62	7.25	0.204	7.05	42.5	7.25
	24.80	69.50	16.62	8.65	0.267	8.38	50.5	7.16
	25.40	70.30	16.62	9.50	0.269	9.23	55.6	7.14
	24.30	90.00	16.62	11.08	0.345	10.74	64.6	7.14
	19.60	101.50	17.81	9.58	0.389	9.19	51.7	7.33
	23.90	115.20	16.62	12.70	0.442	12.26	73.8	7.11

Oxygen Content of Blood.—Any attempt to determine the actual amount of gas existing in the arterial or venous blood of fishes as small as those used in this investigation is beset with difficulties. The few results obtained are recorded in Table II. Attempts to get arterial blood from these fishes were rewarded with little success. Analysis of blood removed from efferent gill arteries of the scup and sea robin showed much less oxygen than could reasonably be expected. It would appear that the syringe used in the operation hastened the circulation through the gill to a point where the blood had not sufficient time to become aërated to the normal degree. A more reliable source of arterial blood is that from the caudal artery, but the fishes used are unsuited for getting blood from such a source. Until a more adequate technique is devised, any statement as to the actual oxygen content of arterial blood in these fishes will have to be postponed. Hall (1930) reported 85 per cent oxygen saturation in mackerel arterial blood. Wastl (1928) found 93 per cent oxygen saturation in carp blood.

With regard to the oxygen content of venous blood, more satisfactory results were obtained. Practically no oxygen was found in the venous blood of the goosefish, toadfish, and puffer.

The gas tensions recorded in Table II for sea robin and toadfish bloods were not determined experimentally but were interpolated from the oxygen dissociation curves for their bloods.

Oxygen Dissociation of Hemoglobin.—Table III, and Figs. 1, 2, and 3 summarize the results of this study. At a carbon dioxide tension of

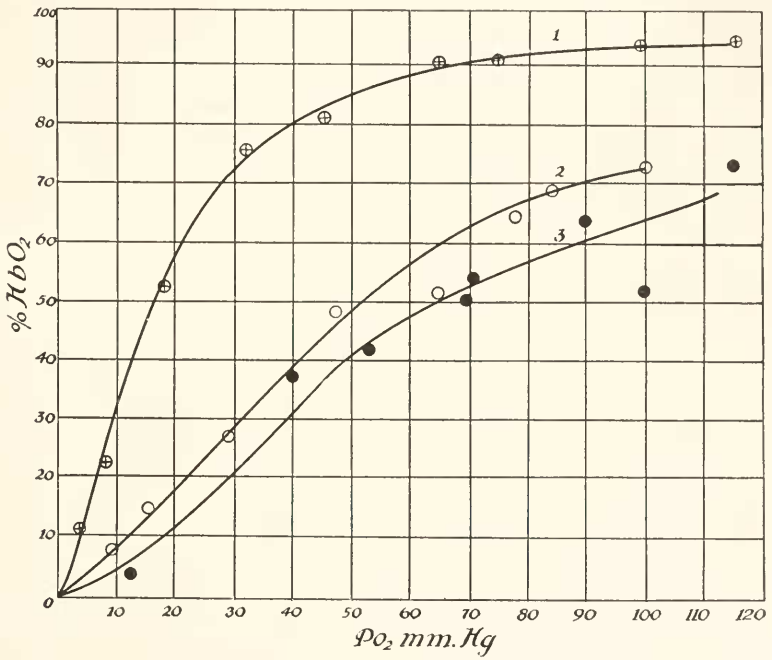

FIG. 3. Oxygen dissociation curves for mackerel blood at 20° C. Curve 1 at 1 mm. carbon dioxide; curve 2 at 10 mm. carbon dioxide; and curve 3 at 25 mm. carbon dioxide tension.

approximately one millimeter toadfish hemoglobin is characterized by a steeper dissociation curve than either sea robin or mackerel. The hemoglobins of the latter appear to act quite the same toward oxygen, at this carbon dioxide tension, except for the fact that sea robin hemoglobin tends to become saturated a little more quickly than mackerel at the higher oxygen tensions. At 10 mm. carbon dioxide tension the dissociation curves for mackerel and toadfish hemoglobins are flattened most remarkably. A still more pronounced flattening is produced at 25 mm. carbon dioxide tension. Of the three hemoglobins the sea

robin's is most affected at the latter carbon dioxide tension. The appearance of the curves at 10 and 25 mm. of carbon dioxide is very interesting. There is a tendency for them to become nearly asymptotic with respect to the abscissa before saturation is complete. This is most noticeable in the curves for toadfish and sea robin hemoglobins. At 10 mm. carbon dioxide tension, in the case of toadfish hemoglobin, the

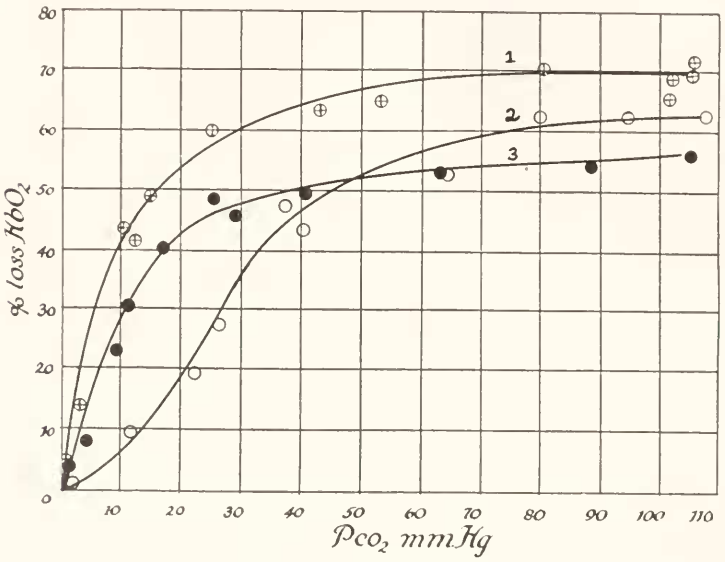

FIG. 4. Effect of carbon dioxide on the oxygen capacity at 20° C. Curve 1 is for sea robin blood; curve 2 for mackerel blood; and curve 3 for toadfish blood.

curve appears to approach a limit at approximately 75 per cent oxygen saturation. At 25 mm. carbon dioxide this same tendency occurs in sea robin hemoglobin at 25 per cent saturation, while toadfish hemoglobin shows this at about 50 per cent saturation. The curves for mackerel hemoglobin do not show any very marked tendency to become asymptotic. In the case of toadfish and sea robin bloods it would appear as if carbon dioxide affected not only the oxygen dissociation constant of the hemoglobin, but, also, that the quantity of oxygen with which the hemoglobin can combine is reduced by the presence of carbon dioxide. Redfield and Mason (1928) have pointed out that such an effect is produced by acid in the case of purified *Limulus* hemocyanin.

Effect of Carbon Dioxide on the Oxygen Capacity.—The peculiar effects of carbon dioxide on the oxygen dissociation curves suggested an investigation of its effect on the so-called oxygen capacity. For this work blood samples were equilibrated with 153 mm. of oxygen and

varying tensions of carbon dioxide. The results are presented in Table IV, and Figs. 4 and 5. As can be seen from the data, carbon

TABLE IV

Effect of carbon dioxide on oxygen capacity. Blood equilibrated at constant P_{O_2}(152 mm.) at 20° C.

Species	P_{CO_2}	O_2-Capacity	O_2-Content	O_2 Dissolved	O_2 Combined	Loss in oxygen capacity	pH
	mm. Hg	*vol. per cent at 0 P_{CO_2}*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	
Toadfish	1.37	6.87	7.22	0.60	6.62	3.64	7.64
	1.54	6.34	6.67		6.07	4.27	7.52
	4.62	6.34	6.50		5.90	6.94	7.42
	9.15	6.34	5.50		4.90	22.70	7.23
	11.75	6.40	5.03		4.43	30.80	7.20
	16.70	6.34	4.38		3.78	40.40	7.08
	25.70	6.34	3.85		3.25	48.80	6.98
	29.30	5.59	3.60		3.00	46.40	6.94
	41.00	6.63	3.98		3.38	49.20	6.82
	63.70	6.40	3.57		2.97	53.60	6.71
	88.00	6.40	3.50		2.90	54.70	6.60
	106.00	6.87	3.58		2.98	56.60	6.48
Sea robin	1.21	7.67	7.92	0.60	7.32	4.50	7.79
	3.79	7.67	7.15		6.55	14.60	7.56
	10.50	7.71	4.95		4.35	43.60	7.30
	12.15	7.67	5.06		4.46	41.80	7.27
	15.25	7.71	4.53		3.93	49.00	7.11
	25.05	7.69	3.67		3.07	60.00	7.06
	43.60	7.15	3.20		2.60	63.60	6.83
	53.00	7.67	3.26		2.66	65.40	6.75
	80.80	8.29	3.04		2.44	70.60	6.68
	102.00	7.15	3.06		2.46	65.60	6.60
	103.00	8.70	3.34		2.74	68.60	6.58
	106.50	7.15	2.82		2.22	69.00	6.57
	107.00	8.15	2.90		2.30	71.80	6.56
Mackerel	2.17	16.43	16.80	0.586	16.21	1.34	7.94
	2.26	16.78	17.15		16.56	1.31	7.84
	12.00	15.64	14.75		14.16	9.90	7.37
	22.50	16.64	14.05		13.46	19.10	7.08
	26.10	14.51	11.05		10.46	27.90	7.21
	37.30	16.78	9.40		8.81	47.50	7.10
	40.80	16.43	9.81		9.22	43.80	7.16
	65.05	16.60	8.35		7.76	53.25	6.95
	80.00	16.78	6.90		6.31	62.60	6.88
	95.00	16.78	7.06		6.47	61.60	6.84
	108.50	16.43	6.96		6.37	61.20	6.82

dioxide affects a very marked loss in oxygen-combining power of the hemoglobins. However, a maximum loss is reached beyond which further addition of carbon dioxide has little or no effect. Sea robin

hemoglobin suffers the greatest loss in oxygen-combining power, the maximum being around 70 per cent, whereas the maximum for toadfish is about 55 per cent. Mackerel hemoglobin has a maximum loss between those for the other two. The data procured seem to corroborate what was already anticipated in a study of the dissociation curves, namely that the ability of the hemoglobins to combine with oxygen is greatly reduced in the presence of carbon dioxide.

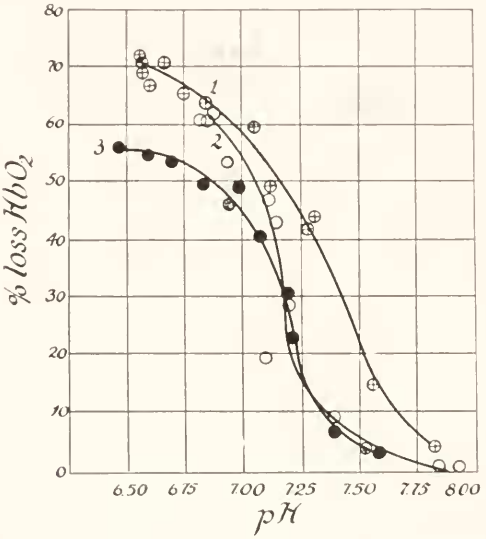

FIG. 5. Effect of pH on the oxygen capacity at 20° C. Curve 1 is for sea robin blood; curve 2 for mackerel blood; and curve 3 for toadfish blood.

Plotting loss in oxygen capacity, or, as designated in Fig. 5, loss in oxyhemoglobin, as a function of pH, yields sigmoid curves for the three hemoglobins. Within a certain range of pH there is a marked loss in oxygen-combining power. Outside this range at either end, within the limits of pH established in these experiments, loss in oxygen-combining power is relatively slight.

Effect of Lactic Acid on Oxygen Capacity.—It was thought advisable to modify the pH of the bloods by other means than the use of carbon dioxide and see if a similar effect on the oxygen capacity could be obtained. Therefore blood samples containing definite concentrations of lactic acid were equilibrated in 153 mm. of oxygen. In this case, of course, no carbon dioxide was added to the gaseous phase in the tonometers. Only the blood of the sea robin was used in these experiments. The results are shown in Fig. 6. A greater loss of oxyhemoglobin was observed at the higher concentrations of lactic acid than was found

when carbon dioxide was used, though the calculated pH was less. However, there may have been some other factor entering in to produce the results, such as the formation of methemoglobin, and, since this was not ascertained, no emphasis should be placed on the magnitude of the results. The main matter of interest is that, in general, the form of the curve is similar to that for the carbon dioxide effect.

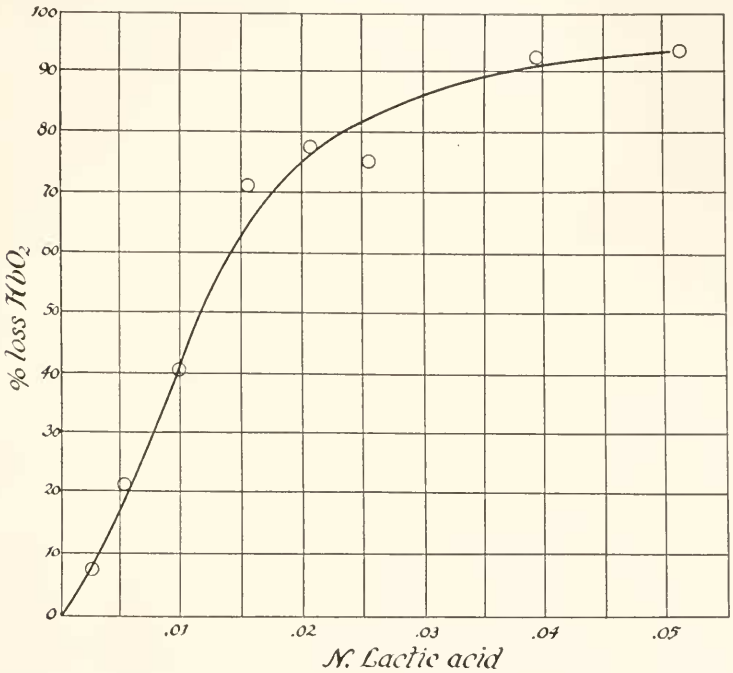

FIG. 6. Effect of lactic acid on the oxygen capacity at 20° C. Curve for sea robin blood only.

B. The Transportation of Carbon Dioxide

Carbon Dioxide Content of Blood.—An attempt was made to determine the amount of carbon dioxide normally present in the circulating blood. The results are recorded in Table II. The bloods of the fishes studied contain relatively little carbon dioxide. The tension even in the venous blood is probably not more than 10 to 15 millimeters.

Carbon Dioxide Absorption of Blood.—The results of this study are presented in Figs. 7, 8, and 9. Of the three bloods examined the toadfish was found to take up the least, and the mackerel to take up the most carbon dioxide. All three curves tend to flatten out above 10 mm. carbon dioxide tension, the flattening being most pronounced in the case of toadfish blood, and least in mackerel. The curve for mackerel blood is quite out of the class of the curves for the other two

fishes. Apparently mackerel blood has a higher available base than toadfish and sea robin bloods.

Christiansen, Douglas, and Haldane (1914) were the first to discover that reduced blood will take up more carbon dioxide than oxygenated blood. This phenomenon has been explained since their work was published by the assumption that oxyhemoglobin is a stronger acid than hemoglobin, and, thus, base is liberated and made available for carbon dioxide when oxyhemoglobin is reduced. The elucidation of the fact is due mainly to the work of Van Slyke and his collaborators at the Rockefeller Institute.

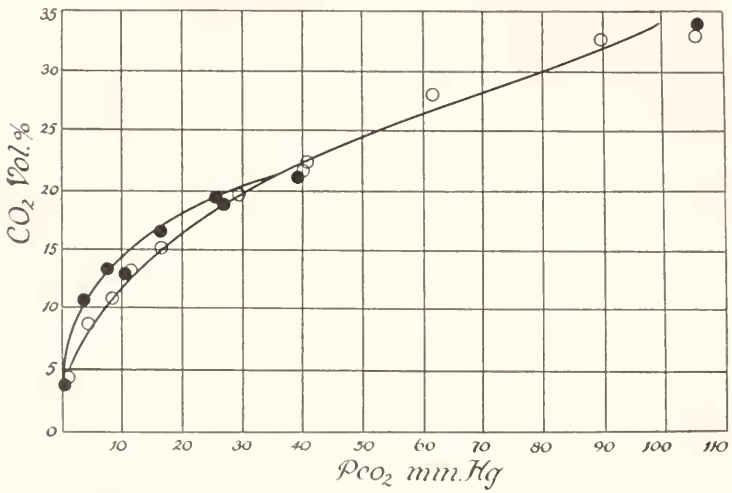

FIG. 7. Carbon dioxide-absorption curves for toadfish blood at 20° C. The dots are for reduced, and the circles for oxygenated blood.

Since it has been demonstrated beyond doubt that reduced blood will take up more carbon dioxide than oxygenated, as far as mammals are concerned, it was thought advisable to determine whether a similar phenomenon could be shown for fish blood. Wastl (1928) has shown such to be the case as far as carp blood is concerned. The results obtained on the bloods of the toadfish, sea robin, and mackerel are shown in the carbon dioxide-absorption curves drawn in Figs. 7, 8, and 9. Toadfish and sea robin bloods show little difference in the ability of reduced and oxygenated to absorb carbon dioxide. Within what appears to be the physiological range of carbon dioxide tension (from analyses of the carbon dioxide content of venous blood), however, reduced blood takes up slightly more carbon dioxide than oxygenated. With respect to mackerel blood the range where this can be demonstrated is considerably greater, and the curves begin to take on the appearance of mammalian carbon dioxide-absorption curves. There are

probably at least two reasons why it is difficult to demonstrate greater carbon dioxide absorption by reduced than by oxygenated blood in the case of the first two fishes: (1) the small amount of hemoglobin present to furnish base in changing from the oxygenated to the reduced state, and (2), the effect of carbon dioxide in reducing the oxygen capacity. One can hardly say he is dealing with oxygenated blood at high carbon dioxide tensions, for under these conditions the oxygenation of the blood is greatly reduced.

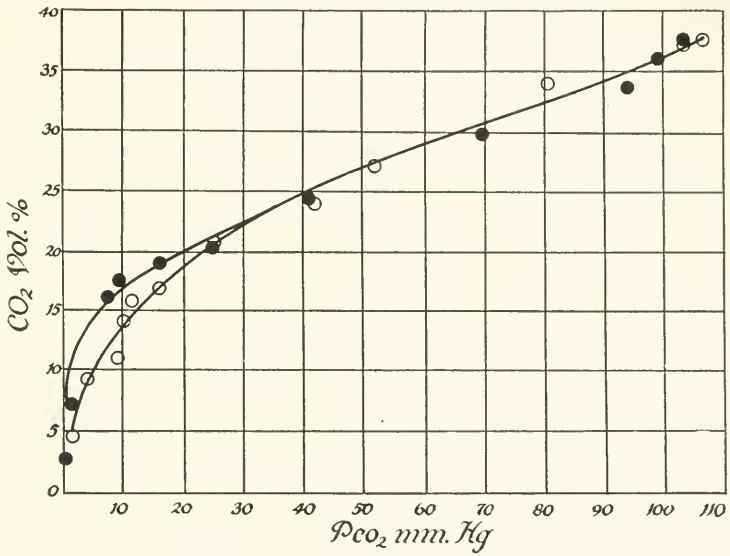

FIG. 8. Carbon dioxide-absorption curves for sea robin blood at 20° C. The dots are for reduced, and the circles for oxygenated blood.

Buffering Ability of Blood.—The $BHCO_3$ concentrations of the bloods have been calculated and the results plotted as a function of pH. Such a procedure will point out their relative buffering ability. The curves obtained are shown in Figs. 10, 11, and 12. In general, within the normal range of pH, reduced blood has a higher concentration of $BHCO_3$ at a given pH than oxygenated. This means that by reduction oxyhemoglobin imparts to the blood a certain protection against change in pH, for a certain added amount of carbon dioxide may be taken up at the same hydrogen ion concentration. Outside the normal pH range the curves for oxygenated and reduced blood tend to converge so that there is practically no difference in the ability of the two states of blood to bind carbon dioxide.

A comparison of the three bloods shows at once that mackerel blood is much better buffered than either toadfish or sea robin. Toadfish

blood is buffered the least of all. However, there is little difference between it and sea robin blood.

Discussion

In the work on the effect of carbon dioxide on the oxygen capacity and on the oxygen dissociation curves a suggestive series of results were obtained. In mammalian hemoglobin the usual effect of carbon dioxide is purely on the oxygen dissociation constant, a simple Bohr effect with no upset in the original oxygen capacity of the particular hemoglobin studied. The hemoglobins of these fishes, however, seem to be affected by carbon dioxide in a manner more complicated. The data suggest that some of the oxygen-binding groups of the hemoglobin

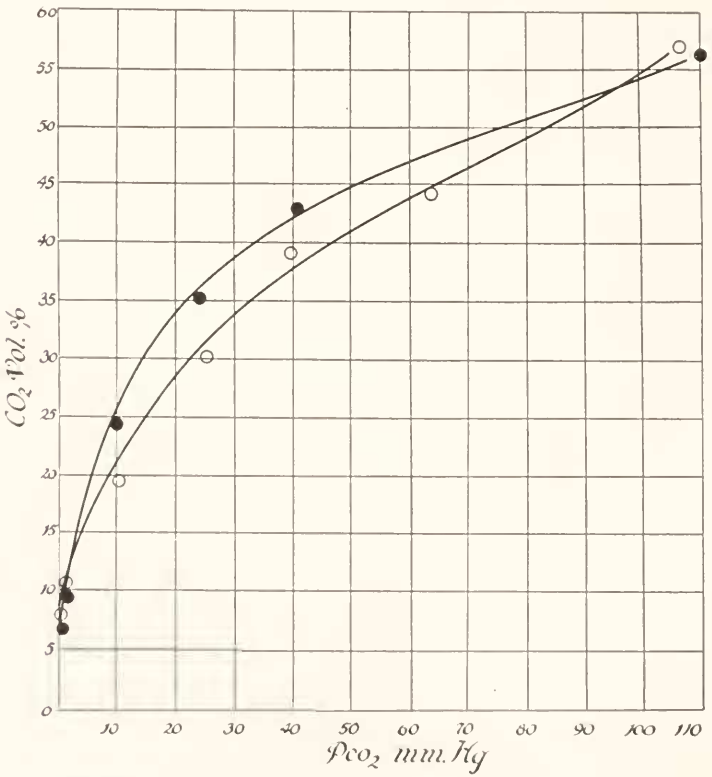

Fig. 9. Carbon dioxide-absorption curves for mackerel blood at 20° C. The dots are for reduced, and the circles for oxygenated blood.

molecule have become inactive. If the hemoglobin molecule combines with four molecules of oxygen, as has been suggested by Adair (1925), it would appear as if carbon dioxide were inactivating one or more of the four prosthetic groups involved in binding oxygen. In other words, it

would look as though the hemoglobin-oxygen reaction were stopping off at one or more of the intermediate compound stages, depending upon how much carbon dioxide is present, instead of the reaction being carried completely through the four theoretical steps presented by Adair.

To illustrate this point attention is recalled to the results on the direct effect of carbon dioxide on oxygen capacity. In the case of toadfish blood there is produced a maximum loss of about 55 per cent

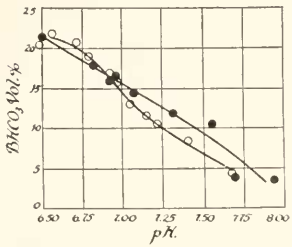

FIG. 10.

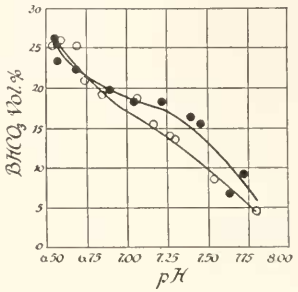

FIG. 11.

FIG. 10. $BHCO_3$: pH curves for toadfish blood at 20° C. The dots are for reduced, and the circles for oxygenated blood.

FIG. 11. $BHCO_3$: pH curves for sea robin blood at 20° C. The dots are for reduced, and the circles for oxygenated blood.

in oxygen capacity in the presence of carbon dioxide, and in sea robin blood about 70 per cent. Mackerel blood under the same conditions experiences a loss of slightly over 60 per cent. Interpreting this situation on the basis of inactivation of oxygen-binding groups, toadfish hemoglobin has two of the four groups inactivated. Thus, allowing for experimental errors, the oxygen capacity drops to a point approximately 50 per cent lower than the original figure for oxygen capacity obtained when the blood was equilibrated in air. Sea robin hemoglobin, and perhaps mackerel, has three of the four groups inactivated. Thus the new figure for oxygen capacity obtained in the presence of considerable carbon dioxide is approximately 75 per cent lower than the original. As has been pointed out previously, these marked drops in oxygen capacity occur at definite ranges of pH.

It will be recalled that reference was made to the peculiar tendency of the oxygen dissociation curves (most marked in the case of those for the toadfish and sea robin) to appear to reach a limit considerably before the 100 per cent oxygen-saturation point was reached. It seems reasonable to suppose that the phenomenon of inactivation of oxygen-binding groups affords an interpretation of this situation.

There is no doubt but that there is danger in carrying the foregoing interpretation too far. The author wishes to emphasize the fact that

the idea of inactivity brought forth in this paper is purely suggestive. Data are far too few to warrant any definite conclusion. If the data really mean that certain prosthetic groups are inactivated, then the oxygen-dissociation curves should present asymptotic relationships from the point of minimum oxygen tension at which the remaining active groups are saturated with oxygen up through oxygen tensions far above those used in these experiments. At the same time the same marked loss in oxygen capacity in the presence of carbon dioxide should be capable of demonstration even though the blood were equilibrated in pure oxygen. It is regretted that higher oxygen tensions were not

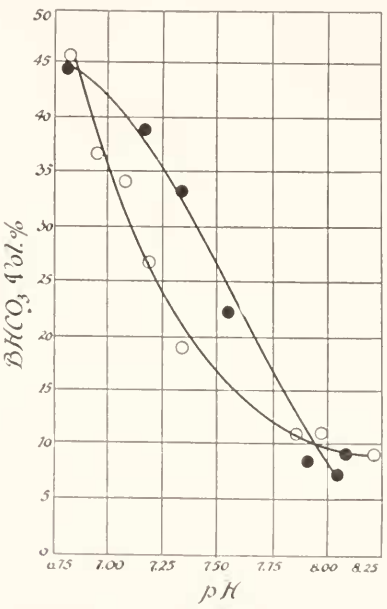

FIG. 12. $BHCO_3$: pH curves for mackerel blood at 20° C. The dots are for reduced, and the circles for oxygenated blood.

used, for it seems that if such had been the case the idea of inactivity would have had either a stronger case in its favor or been thrown out entirely. It may be that the entire situation is a greatly exaggerated Bohr effect, and all that is necessary is higher oxygen tensions to bring back the original oxygen capacity.

If the idea presented in this paper proves upon further experimentation to be correct, we have before us a means of furthering the study of Adair's theory of the combination of oxygen with hemoglobin.

Aside from the physical chemistry of fish hemoglobin, the relation of the data presented to the life of the fish is interesting. We find a correlation between the transportation of oxygen and the environment and

habits of the fishes. The sluggish fishes have bloods of low oxygen capacity, and the active of high capacity. Thus, there is evidence of adjustment between oxygen capacities and oxygen requirements, for, as Hall (1929) has shown, the sluggish fishes do not consume as much oxygen per unit time as the active. Further evidence of adjustment is shown in the form of the oxygen dissociation curves at low carbon dioxide tensions. The toadfish hemoglobin, under these conditions, becomes saturated with oxygen at a much lower tension than is the case with the other two fishes. This may partially explain the ability of this fish to live in water of abnormally low oxygen tension (Hall, 1930). On the other hand, mackerel hemoglobin, in the presence of 1 mm. of carbon dioxide, requires a considerably greater tension of oxygen to become saturated than is the case for the other fishes studied. This may account in part for the great susceptibility of the mackerel to asphyxiation. Hall (1930) found that a mackerel requires a strong circulation of oxygen-loaded sea water over its gills in order to prevent excessive oxygen-unsaturation of its blood, and consequent death due to asphyxia.

The high sensitivity of all three hemoglobins to carbon dioxide indicates that they are adjusted to an environment of low carbon dioxide tension, such as the gills offer. Any one of the fishes examined would experience considerable difficulty in getting sufficient oxygen were the environment in which its gills are bathed loaded with free carbon dioxide. Krogh and Leitch (1919) and Redfield *et al.* (1926) have alluded to the apparent adjustment of the oxygen dissociation curves to the environment and habits of animals. Krogh and Leitch offered such a conclusion after working on the blood of fishes, while Redfield and collaborators came to the same conclusion after investigating certain bloods containing hemocyanin. The work presented here corroborates their evidence.

With regard to the transportation of carbon dioxide by the blood of marine fishes, this investigation shows that the amount bound by the various bloods is not the same for all species. Directionally the same differences occur as were found in the ability of the bloods to combine with oxygen. Mackerel blood is not only able to bind greater quantities of oxygen, but is also able to bind greater quantities of carbon dioxide than either toadfish or sea robin blood. This strongly suggests that the greater concentration of hemoglobin in mackerel blood is responsible for the difference noted. It is known that hemoglobin affects the height and slope of carbon dioxide-absorption curves. This has been pointed out by Peters, Bulger, and Eisenman (1924) and others. The writer, too, found that anaemic fish blood would not take up as

much carbon dioxide as normal blood of a species. It is generally recognized that hemoglobin plays an important rôle in the transportation of carbon dioxide. This has been shown by Van Slyke (1921) and many other workers. However, just how close a relationship there is between the hemoglobin concentration and the ability of fish blood to carry carbon dioxide cannot be stated at this time.

The greater concentration of hemoglobin in mackerel blood may also account for the fact that it is easier to demonstrate greater carbon dioxide absorption by its reduced than by its oxygenated blood, than to do it with either toadfish or sea robin blood.

The small amount of carbon dioxide found in the circulating blood of these fishes is in agreement with the findings of Kokubo (1930) for certain other marine species. At the same time the relatively poor buffering ability of their blood agrees with data on other forms presented by Collip (1920), Wastl (1928), and Kokubo (1930). The facts that there is little carbon dioxide normally present in the blood of these fishes, and that it is poorly buffered against carbon dioxide, again sug-

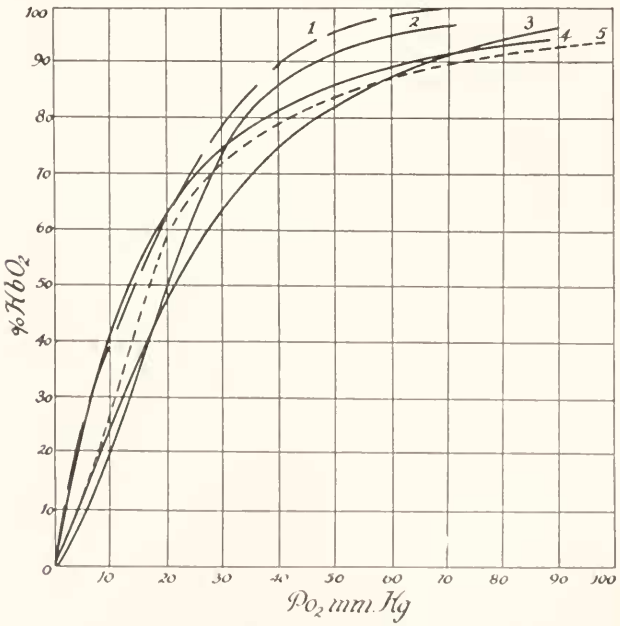

FIG. 13. Comparative oxygen dissociation curves. Curve 1 is for toadfish blood at 20° C. and 1 mm. carbon dioxide; curve 2 for human blood at 37.5° C. and 20 mm. carbon dioxide; curve 3 for turtle blood at 25° C. and 40 mm. carbon dioxide; curve 4 for carp blood at 18° C. and 30 mm. carbon dioxide; and curve 5 for mackerel blood at 20° C. and 1 mm. carbon dioxide tension.

gest an adjustment of the bloods to sea water. There is a low carbon dioxide tension in the gill of a marine fish, a fact necessarily correlated

with the low carbon dioxide tension in sea water. At the same time, because of low metabolic rate, a fish produces relatively small quantities of carbon dioxide. Mammalian blood must, by virtue of the high alveolar carbon dioxide tension and the greater metabolic activity on the part of the animal, be prepared to handle larger quantities of carbon dioxide than the blood of a fish. The situation as it stands appears to point to adjustment on the part of both fish and mammal blood to the particular physiological, morphological, and ecological differences that concern the two types of vertebrates.

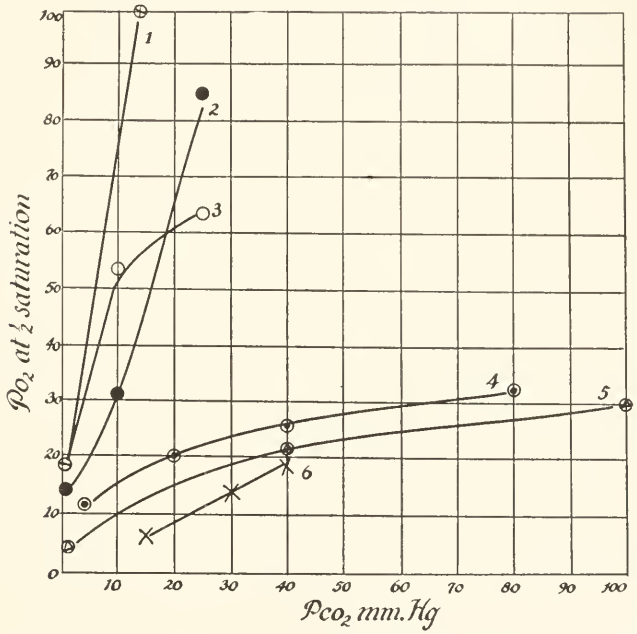

FIG. 14. Effect of carbon dioxide on the "unloading tension" (Po_2 when blood is half saturated) of various vertebrate bloods. Curve 1 is for sea robin blood at 20° C.; curve 2 for toadfish blood at 20° C.; curve 3 for mackerel blood at 20° C.; curve 4 for human blood at 37.5° C.; curve 5 for turtle blood at 25° C.; and curve 6 for carp blood at 18° C.

The calculated pH of fish blood is less than that of sea water. One may wonder how the blood maintains a lower pH. The facts that the blood is poorly buffered, and that it maintains a carbon dioxide tension normally higher than that of sea water probably account for the lower pH.

In comparing the data presented in this paper with similar data on other vertebrates, several interesting differences are brought out. In Fig. 13 a family of oxygen dissociation curves is shown. Conditions

have been chosen in such a manner as to make the curves fairly near alike. The oxygen dissociation curve for human blood has been constructed from the data of Bock, Field, and Adair (1924); that for the turtle from Southworth and Redfield's (1926) work; and that for the carp from Wastl's (1928) data. The most noticeable thing about these curves is the diversity of conditions under which they were established. The only way one can make them resemble each other fairly closely is to establish them under widely different conditions of temperature and carbon dioxide tension.

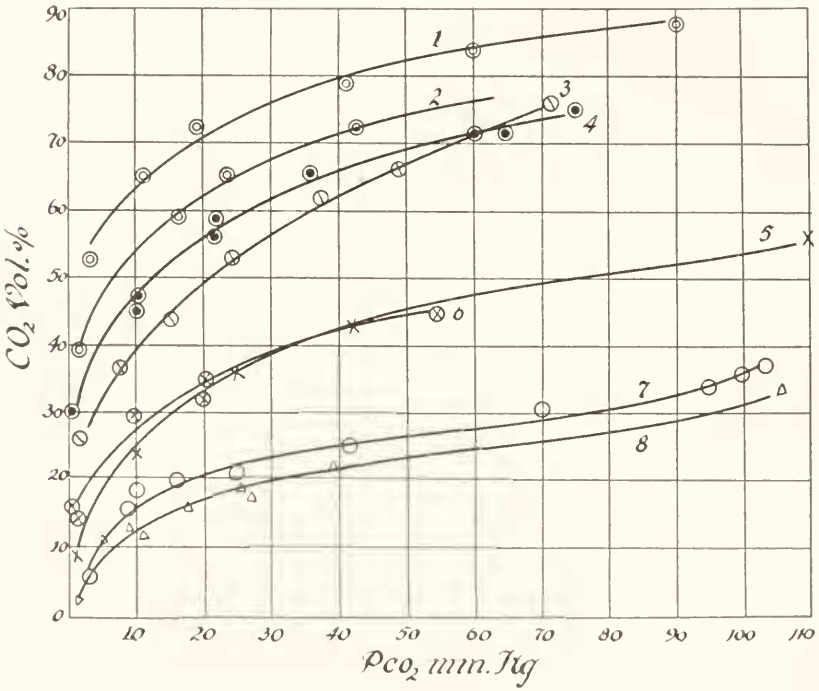

FIG. 15. Comparative carbon dioxide-absorption curves for reduced blood (except for turtle). Curves 1 and 2 are for turtle blood at 25° C.; curve 3 for human blood at 15 °C.; curve 4 for frog blood at 15° C.; curve 5 for mackerel blood at 20° C.; curve 6 for carp blood at 18° C.; curve 7 for sea robin blood at 20° C.; and curve 8 for toadfish blood at 20° C.

In order to show how these same bloods are affected differently by carbon dioxide Fig. 14 has been constructed. One can see at once that the effect of carbon dioxide on marine fish blood is profoundly different from its effect on either human, turtle, or carp blood.

The foregoing comparisons point out well the specificity of hemoglobin in nature that Barcroft (1928) stresses. The significance of specificity is great. Were all hemoglobins alike many animals would

not be able to exist under the conditions of their environment, or of their assumed structural and functional characteristics.

For the purpose of showing the differences between the carbon dioxide-absorption curves of various vertebrate bloods Fig. 15 is presented. The data plotted are for reduced blood, except in the case of the turtle. The curves for human, frog, and carp bloods have been constructed from the data of Wastl and Seliškar (1925), and Wastl (1928); and those for the turtle from Southworth and Redfield's (1926)

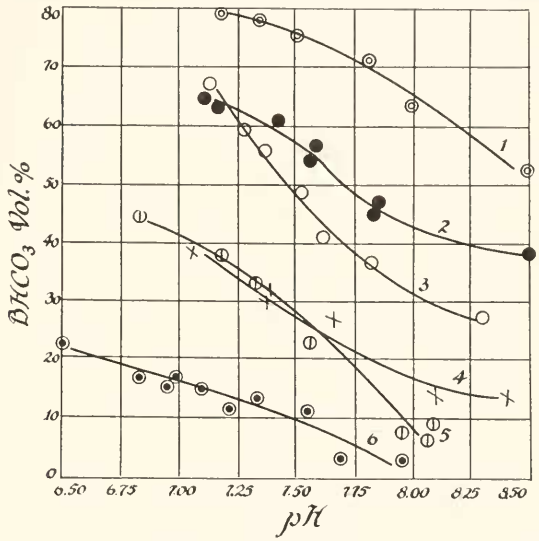

FIG. 16. Comparative $BHCO_3$: pH curves for reduced blood (except for turtle). Curve 1 is for turtle blood at 25° C.; curve 2 for frog blood at 15° C.; curve 3 for human blood at 15° C.; curve 4 for carp blood at 18° C.; curve 5 for mackerel blood at 20° C.; and curve 6 for toadfish blood at 20° C.

data. The curves show that the blood of fishes is characterized by a relatively weak, those of the frog and turtle by a relatively strong, and that of the human by a more or less intermediate carbon dioxide-combining power. Human blood yields the steepest carbon dioxide-absorption, which means that it is buffered the best. These curves have been plotted at as near the same temperature in all cases as possible, since it has been shown by Warburg (1922), Stadie and Martin (1924), and Cullen, Keeler, and Robinson (1925) that temperature affects the carbon dioxide-combining power of blood.

In order that the buffering ability of several vertebrate bloods might be compared Figs. 16 and 17 were constructed. Data other than the author's have been taken from the previously mentioned sources and the pH or cH calculated on a basis comparable to the calculations made for marine fish blood. In Fig. 16 the $BHCO_3$: pH relationships are

shown; in Fig. 17 the $10^{-8} \times cH : Pco_2$ relationships. In the first figure the more nearly parallel the curve runs with respect to the abscissa the more poorly the blood is buffered. The results here indicate that toadfish blood is the poorest buffered, while human blood is the best buffered. There appears to be little difference in the other bloods. In the second figure the steeper the curve is, the poorer the blood is buffered against carbon dioxide. The results obtained here indicate that toadfish and sea robin blood are relatively poorly buffered, while frog, turtle, and human blood are relatively well buffered. Carp and mackerel blood are more or less intermediate with respect to the others, resembling, however, the bloods of the higher vertebrates slightly more than those of the toadfish and sea robin.

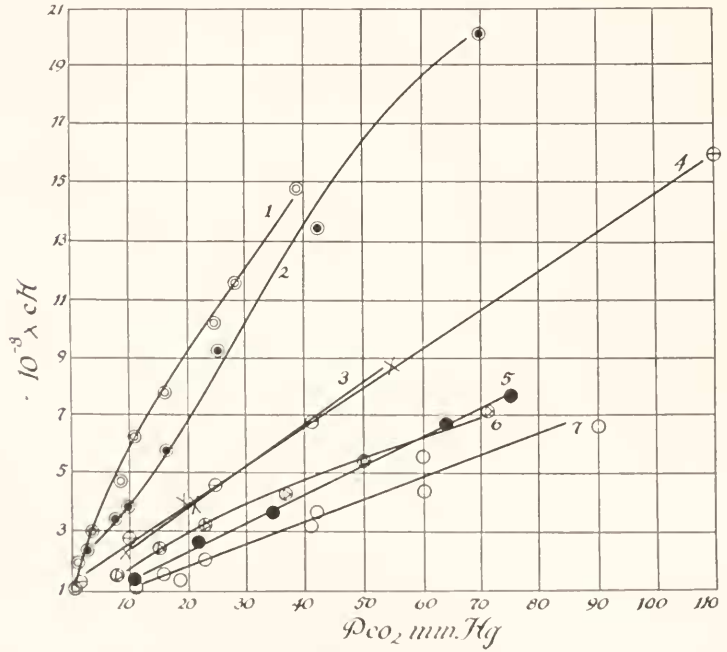

FIG. 17. Comparative $10^{-8} \times cH : Pco_2$ curves for reduced blood (except for turtle). Curve 1 is for toadfish blood at 20° C.; curve 2 for sea robin blood at 20° C.; curve 3 for carp blood at 18° C.; curve 4 for mackerel blood at 20° C.; curve 5 for frog blood at 15° C.; curve 6 for human blood at 15° C.; and curve 7 for turtle blood at 25° C.

There is another point of interest about Figs. 16 and 17. Regardless of the slope of the curves, at any given pH the bloods do not have the same $BHCO_3$ content; likewise at any given cH they are not subjected to the same carbon dioxide tension. This may be explained by the fact that the carbon dioxide-absorption level is quite different for

the different bloods. The higher the level at a given carbon dioxide tension the more the hydrogen ion concentration is displaced in the alkaline direction. Southworth and Redfield (1926) have shown that as far as turtle blood is concerned the characteristically high level of the carbon dioxide-absorption curve is due to high $BHCO_3$ in the plasma and the relatively small amount of hemoglobin present to act as an acid in dissociating carbon dioxide from its salt. Perhaps the same thing holds true for frog blood. It is interesting to note that in the case of toadfish and sea robin blood the dissociation of carbon dioxide is quite complete even though there is a low hemoglobin concentration.

The differential buffering ability of the bloods may possibly be explained on the basis of the nature of the adjustments that vertebrates have undergone in going from an aquatic to a terrestial environment. The acquirement of lungs and a higher rate of metabolism has made necessary a greater buffering defense.

Summary

1. The oxygen capacities of marine fish bloods are quite different for different species. The greatest difference is between the typically sluggish and active forms, the former having bloods of low, and the latter bloods of high oxygen capacity. There is a general correlation between oxygen capacity and corpuscle count, corpuscle volume, and iron content.

2. Studies on the oxygen dissociation curves of marine fish hemoglobin, and on the effect of carbon dioxide on the oxygen capacity have brought forth the suggestion that the effect of carbon dioxide on the hemoglobins of these fishes is not solely on their oxygen dissociation constants, but that there is an inactivation of certain of the prosthetic groups concerned in binding oxygen in the hemoglobin molecule, causing a marked decrease in oxygen-combining power of the bloods. The most marked evidence of inactivation occurs at definite ranges of carbon dioxide tension and pH for the different bloods.

3. The carbon dioxide-combining power of fish bloods appears to be correlated with hemoglobin concentration. Mackerel blood with high hemoglobin absorbs more carbon dioxide than toadfish blood, which has a low hemoglobin concentration.

4. Reduced fish blood will absorb slightly more carbon dioxide than oxygenated blood. For sea robin and toadfish bloods the range of carbon dioxide tension where this can be demonstrated is short, being between about 2 and 25 mm., while it is longer for mackerel blood, being about 2 to 95 mm.

5. There is a differential buffering ability shown by these bloods, mackerel blood being buffered the best and toadfish the poorest.

6. Comparative studies of vertebrate bloods strengthen the idea of specificity of hemoglobins. Those of the marine fishes are far more sensitive to carbon dioxide than those of the carp, turtle, and human.

7. Comparative studies on carbon dioxide transportation show that turtle and frog bloods have a relatively great, fishes a relatively small, and human blood a more or less intermediate carbon dioxide-combining power. The bloods also vary considerably in their buffering capacity, human blood having the greatest and toadfish blood the least.

8. The general results of this investigation point to an adjustment on the part of the blood of marine fishes to a sea-water environment, and the habits or characteristics of the fishes. At the same time the comparative studies indicate marked differences between the bloods of fishes and terrestrial vertebrates. These differences can perhaps be accounted for on the basis of the new morphological and physiological features that terrestrial vertebrates have acquired, along with change in environment, which have made necessary correlative changes in the respiratory function of the blood.

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